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RNasin® Plus RNase Inhibitor

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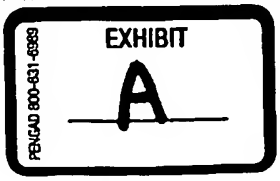
1. **What is RNasin® Plus RNase Inhibitor?**
   RNasin® Plus RNase Inhibitor is a recombinant mammalian RNase inhibitor expr soluble protein in *E. coli*. Through natural amino acid diversity RNasin® Plus RNa has increased resistance to oxidation when compared to the human protein.

2. **Will RNasin® Plus RNase Inhibitor work better than native or Recombin RNasin® Ribonuclease Inhibitor?**
   RNasin® Plus RNase Inhibitor is naturally more resistant to oxidative stress, incr overall efficacy. As an added feature RNasin® Plus displays continued inhibition higher temperatures. Also, RNasin® Plus is expressed by *E. coli* as a soluble pro allowing easy purification by a combination of ion exchange and hydrophobic int chromatography. No direct affinity chromatography is required. This new proces >90% pure protein with no *E. coli* RNase carryover. However, the mechanism o remains the same for RNasin® Plus and native or Recombinant RNasin® Inhibito mechanism is an inhibition of eukaryotic RNases via stoichiometric 1:1 noncoval of the RNasin® Plus RNase Inhibitor to an RNase.

3. **How is RNasin® Plus RNase Inhibitor more resistant to oxidative stress native or recombinant RNasin®?**
   Two cysteines in the human protein have been identified as especially sensitive and react by forming a disulfide that can block the active site of the inhibitor (1) Plus RNase Inhibitor, through natural amino acid diversity, lacks the ability to fo blocking disulfide.



4. **What are the characteristics of RNasin® Plus RNase Inhibitor?**
During development of the RNasin® Plus RNase Inhibitor Promega scientists disc continued inhibition of RNases even above the normal denaturation temperature RNasin® Plus molecule. A mixture of RNasin® Plus and a pure RNase, like RNase heated to at least 70°C for 15 minutes, and the RNase A activity does not return cooling to normal temperatures, such as those used in the RT step of RT-PCR. R has been demonstrated to work in this manner with a complex mixture of RNase in a rat liver protein extract (Sigma Cat.# L1380). Rat liver is known to contain ribonucleases (2). No detectable RNase activity, as determined by RT-PCR, is ob when a mixture of rat liver RNases and RNasin® Plus RNase Inhibitor is heated t 15 minutes followed by the addition of 100ng or 10ng of template RNA and incul an additional hour at 37°C.

5. **How can I use the characteristics of RNasin® Plus RNase Inhibitor to be protect my RNA template?**
Many protocols, including those for ImProm-II™ Reverse Transcription System r an initial thermal denaturation of the RNA template of interest in the presence o reverse transcription primers for 5–10 minutes at 70°C followed by a quick chill step denatures secondary structure in the RNA template, allowing greater sensit PCR. In light of the new activities identified for RNasin® Plus RNase Inhibitor, inl now be added at this step to protect the RNA template during thermal denaturat RNases that were present during the thermal denaturation will be inactivated; h more RNase inhibitor should be added during full RT reaction assembly in the ev additional exogenous RNases are inadvertently added to the reaction from pipet components or other sources.

6. **How do the characteristics of RNasin® Plus RNase Inhibitor relate to hig temperature reverse transcriptase (RT) reactions?**
The characteristics of RNasin® Plus RNase Inhibitor allow you to set up your higl temperature first-strand synthesis reactions and take them to reverse transcript reaction temperatures above 50°C. This gives researchers RNase protection whe transcribing RNA templates with high secondary structure.

7. **What applications are compatible with RNasin® Plus RNase Inhibitor?**
RNasin® Plus RNase Inhibitor has been tested in RT-PCR and is compatible with such as AMV, M-MLV and ImProm-II™ Reverse Transcriptases or *Taq* and *Tfl* DN Polymerases. RNasin® Plus RNase Inhibitor has also been tested and found to b with quantitative, real-time RT-PCR reactions in a TaqMan® Assay. The new inhi compatible with the Riboprobe® System for in vitro transcription using the T3, T RNA Polymerase. RNasin® Plus RNase Inhibitor can also be used with Wheat Ge and Rabbit Reticulocyte Lysate for in vitro translation from an RNA template, as TNT® Wheat Germ and TNT® Reticulocyte Lysate System for coupled in vitro transcription/translation.

References

1. Kim, B.M., Schultz, L.W. and Raines, R.T. (1999) Variants of ribonuclease inhibit resist oxidation. *Protein Sci.* **8**, 430–4.

2. Zhao, W. *et al.* (1998) Ribonucleases from rat and bovine liver: Purification, spe structural characterization. *Biochim. Biophys. Acta* **1384**, 55-65.